

Modulation of the *Gloeobacter violaceus* Ion Channel by Fentanyl: A Molecular Dynamics Study

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Supporting Information

ABSTRACT: Fentanyl is an opioid analgesic, which is routinely used in general surgery to suppress the sensation of pain and as the analgesic component in the induction and maintenance of anesthesia. Fentanyl is also used as the main component to induce anesthesia and as a potentiator to the general anesthetic propofol. The mechanism by which fentanyl induces its anesthetic action is still unclear, and we have therefore employed fully atomistic molecular dynamics simulations to probe this process by simulating the interactions of fentanyl with the *Gloeobacter violaceus* ligand-gated ion channel (GLIC). In this paper, we identify multiple extracellular fentanyl binding sites, which are different from the transmembrane general anesthetic binding sites observed for propofol and other general anesthetics. Our simulations identify a novel fentanyl binding site within the GLIC that results in conformational changes that inhibit conduction through the channel.

The identification of opioid molecules, such as morphine and fentanyl, which cause desensitization to painful stimuli by acting upon G-protein-coupled receptors (GPCRs), has allowed great advances in modern medicine and invasive surgery. Other uses have also been identified for opioids such as fentanyl, i.e., in the potentiation of the general anesthetic propofol and as the main anesthetic component.^{1,2} However, the mechanism of the anesthetic action of opioids remains unclear. Here, we have investigated target ion channels, specifically the Cys-loop family of pentameric ligand-gated ion channels (pLGICs). These proteins are sensitive to neurotransmitters from the presynaptic axon terminal and are hence major drug targets.³ Anesthetics are known to modulate both cation- and anion-permeable channels, such as the γ -Aminobutyric acid type A (GABAA) and nicotinic acetylcholine receptors (nAChRs),^{4,5} but high-resolution structures of eukaryotic receptors have proved to be challenging to obtain. Crystal structures of the bacterial homologue (GLIC) have been obtained at reasonably high resolutions (2.4–4 Å),⁶ which allows researchers the opportunity to study the modulation of pLGIC at the atomic level. This family of ion channels was chosen on the basis of evidence from other studies that show an “anesthetic binding pocket” that general anesthetics have been shown to occupy.^{7,8} The analgesic/anesthetic drug ketamine has also been shown to bind to the

GLIC structure⁹ in a different, extracellular binding site compared to that of general anesthetics.

Figure 1 shows the structure of fentanyl and the transmembrane domain (TMD) of the GLIC. The TMD consists of

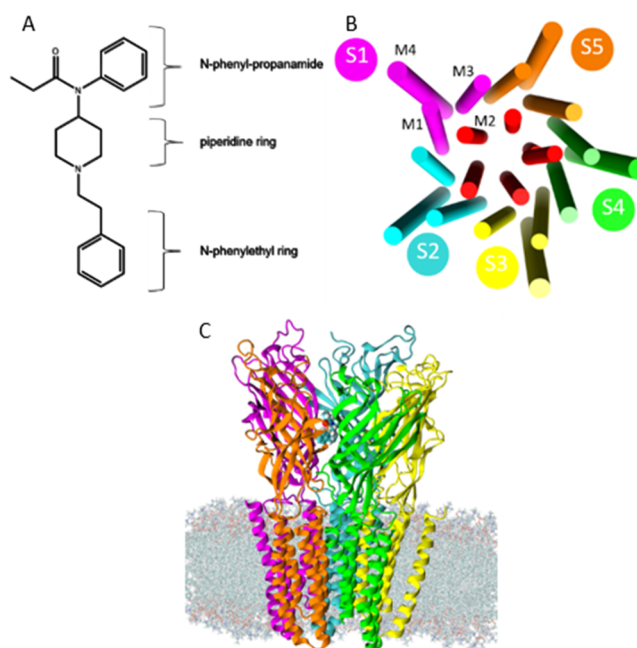


Figure 1. (A) Chemical structure of fentanyl. (B) Graphical representation of the transmembrane α -helical domains showing the five symmetric subunits and the ion-conducting M2 helices (red). (C) GLIC structure in the membrane environment with fentanyl (spheres) in its intersubunit site.

four α -helices that span the entirety of the cell membrane (M1–M4) in which the GLIC is imbedded. The M2 α -helices are oriented toward the center of the pore that forms the ion-conducting, fully hydrated channel in the open state. The evidence of anesthetics interacting with and modulating these channels^{10–12} provides an excellent starting point for the exploration of the anesthetic properties of fentanyl and how it can potentiate other general anesthetics.

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The GLIC open state structure at atomic resolution (2.4 Å) published by Sauguet et al.⁶ and the previous simulation studies of anesthetics interacting with ion channels^{13–15} provide us with the opportunity to compare the effect of fentanyl on a GLIC structure with the binding and modulation of the channel by general anesthetics. We have employed molecular dynamics (MD) simulations and efficient end state free energy calculations to probe the interactions between fentanyl and the GLIC. Our simulations reveal that fentanyl occupies multiple extracellular binding sites similar to those observed for ketamine,⁹ which lead to conformational changes within the M2 helix domain, causing pore closure and dehydration resulting in a nonconductive state.

We initially performed three separate 500 ns MD simulations on the pure GLIC structure inserted into a 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) lipid bilayer⁶ as a reference, followed by three 500 ns simulations in which four fentanyl molecules were added to the simulation box in each system, where the equilibrated pure GLIC system before its production run was used as the starting structure. From the pure GLIC simulation, root-mean-square deviations (RMSDs) were calculated to assess whether the protein structure was stable within the membrane (Tables S1 and S2). Several binding sites were identified, and molecular mechanics Poisson–Boltzmann surface area (MMPBSA)¹⁶ calculations were performed to assess the strength of binding at each site. Figure 2 shows the residues in the fentanyl binding site, which

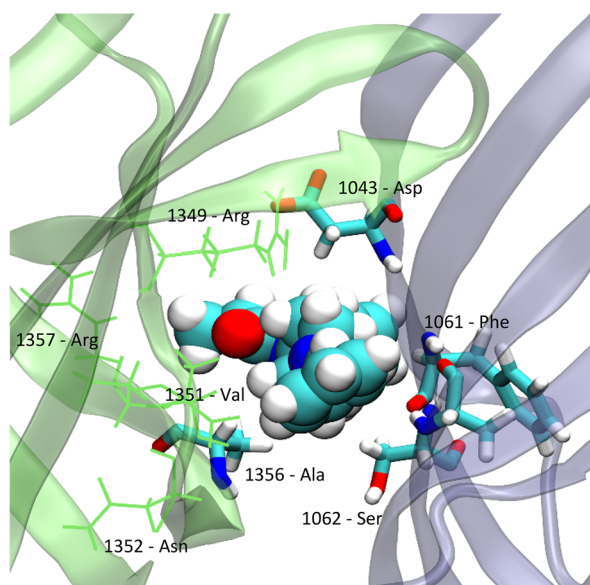


Figure 2. Detailed view of fentanyl binding in its intersubunit extracellular site. Fentanyl is shown in van der Waals sphere representation, with the strongest binding residues shown in licorice representation and additional residues shown in stick representation.

has a calculated binding free energy of -27.35 ± 0.06 kcal/mol (binding free energies and residues of other sites are listed in Tables S3–S8). Binding contributions from the ligand and the binding site residues are listed in Table S9.

The fentanyl molecule showing the strongest binding energy initially interacts with the hairpin loop located at the top of the extracellular domain for ~20 ns before diffusing into the channel, where it then enters the binding pocket (Table 1). This binding pocket is situated between two subunits in the extracellular domain above the lipid headgroup region.

Table 1. Fentanyl Binding Sites in Order of Binding Free Energy

extracellular binding site	residence time (ns)	binding free energy (kcal/mol)
1	450	-27.35 ± 0.06
2	88.3	-18.88 ± 0.18
3	78.1	-12.29 ± 0.17
4	63.4	-12.00 ± 0.20
5	57.9	-10.73 ± 0.15
6	39.3	-8.29 ± 0.11
7	9.7	-6.18 ± 0.22

Fentanyl initiates binding at ~40 ns in one simulation and at ~50 ns in another, and they both remain within the binding site for the rest of the simulation time, totaling 460 and 450 ns of binding time, respectively, with little structural deviation from the binding conformation (RMSD = 1.7 ± 0.8 Å), indicating that this is a very stable binding site. Secondary structure calculations show that there is no appreciable change in the secondary structure of the binding pocket (Figure S2) before or after fentanyl binding. Root-mean-square fluctuation calculations (Figure S8) for the two subunits that form the binding site show that fentanyl causes an increased fluctuation in S5 but stabilizes loop C, which forms the top of the binding site. At the other binding sites, fentanyl dissociated and diffused into the membrane domain where it remained for the rest of the simulations.

To assess the effects of binding on the function of the GLIC, we computed the change in the number of pore water molecules, using an in house python script utilizing the MDAnalysis python toolkit.¹⁷ The definition of pore water molecules is given in the section 1 of the Supporting Information. This methodology has previously been used to analyze hydrophobic gate formation in other channel proteins.¹⁸ Our analysis of the pure system confirmed that the channel was in the open state (Figure 3).

To identify visually the formation of the hydrophobic gate, VMD¹⁹ was used to identify the gating residues. From this information, we were able to identify residues 233-Ile and 240-Ile as the residues forming the hydrophobic gate and causing dehydration of the M2 pore, which is consistent with the GLIC

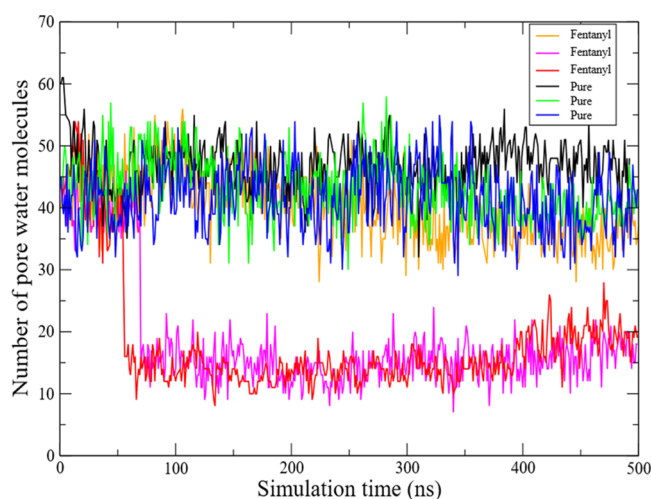


Figure 3. Plot highlighting the change in the number of pore water molecules within the M2 helix channel pore when fentanyl is bound.

hydrophobic gating residues identified in other studies.^{7,20,21} This gating mechanism is similar to that for acetylcholine receptors that rely on M2 helix rotations to control ion conduction.²²

Our analysis clearly shows the formation of a hydrophobic gate within the pore that is consistent with that observed for general anesthetics. In addition, the M2 pore radius of the pure simulation is less contracted than that of the fentanyl system, instead resembling the open state of the crystal structure (Figure 4). As this change of state observed in the fentanyl

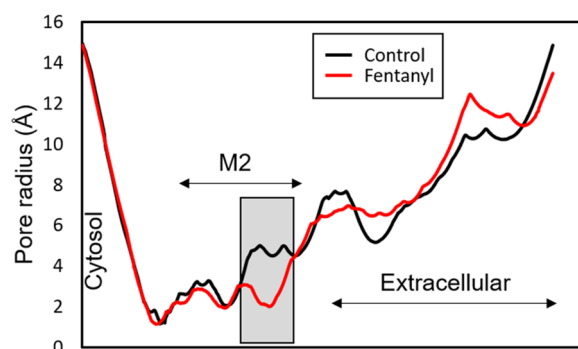


Figure 4. M2 pore radius calculations for the pure/control and fentanyl simulations averaged over the trajectories. The gray box represents the hydrophobic gate region. Calculations performed using HOLE.²³

simulation is not observed within our control simulation, we suggest that hydrophobic gate formation is caused by the binding of the fentanyl molecule at ~40 ns.

To determine if the formation of the hydrophobic gate inhibited ion conduction through the channel, we have performed several applied electric field simulations (details given in section 1 of the Supporting Information). Ion conduction was observed in the pure GLIC system after ~60 ns at a transmembrane voltage of 270 mV, but no conduction was observed in the fentanyl-bound system, in which ions remained above the hydrophobic gate residues (Figure S10). Fentanyl remained bound during the course of these simulations.

The conformational change within the TMD associated with anesthetic binding is an ~10° tilt in the upper region of the M2 helix, causing an “iris-like” contraction.^{7,20,24} To analyze the effect of fentanyl binding on the geometry of the M2 helix, we employed the TRAJELIX²⁵ module as incorporated in the Simulaid analysis program,²⁶ and the Bendix²⁷ plugin for VMD. We used this methodology to calculate the global helix x , y , and z tilt angles; the turn angle per residue; the local helix tilt; the helix rotation; and the angle of curvature for each M2 transmembrane helix over the course of the 500 ns trajectories. Full results of this analysis can be found in the Supporting Information. Figure 5 shows the helix rotation for one of the five M2 helices, as they were all relatively similar (see also Figures S3–S7). We consistently observed a rotation of 10° with a small degree of local helix tilting, which suggests a method of pore contraction different from that observed in binding general anesthetics.^{7,20,24} From the curvature analysis, we observe that the general trend for the pure system is perturbed by fentanyl binding. The most noticeable increase in curvature is observed in the first 10 residues of the helix, which include the hydrophobic gate region.

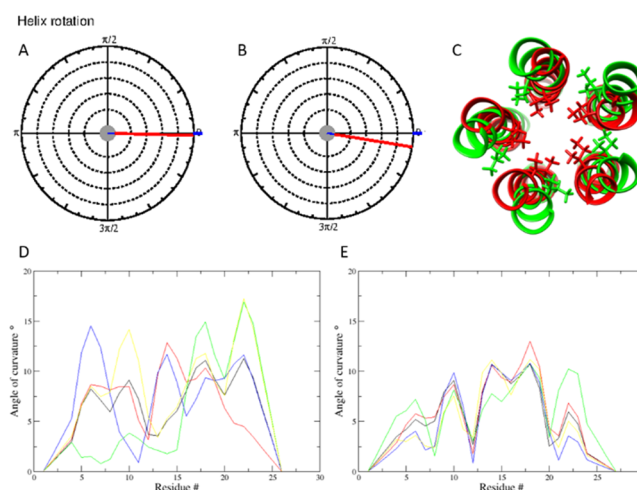


Figure 5. (A) Dial plot indicating the helix rotation of the S3M2 helix in the pure GLIC system. (B) Dial plot indicating the helix rotation of the S3M2 helix in the fentanyl-bound system. (C) Overlapped structures of the M2 TMD, with green indicating an open channel (fentanyl not bound) and red indicating a closed channel (fentanyl bound), with Ile gating residues shown in stick representation (rendered with UCSF Chimera²⁸). (D and E) Helix curvature plots for fentanyl and pure simulations, respectively, where each line represents one subunit.

Although a full signaling pathway or mechanism of action for the action of neurotransmitters and drugs on pLGICs remains unclear, several studies have proposed conformational changes in certain regions of the channels. Salt bridge perturbation has been shown to be significant in the gating process for nicotinic and GABA receptors, as well as the GLIC.^{29,30} In our fentanyl-bound simulations, we have computed the distance between the center of mass (COM) of the 32-Asp and 192-Arg salt bridge (Figure S9), which was shown to break after fentanyl binding. Loop C was also stabilized in our simulations (Figure S8), which is also seen in various pLGIC ligand studies where loop C is stabilized in structurally different conformations.³¹

From this analysis, we can hypothesize that the difference in helix conformational change is due to the extracellular binding site, which has no direct interaction with the helices themselves. In contrast, the anesthetic binding site, which is either between TMD subunits or within the four helices of the subunits themselves,^{32–34} does directly interact with the M2 pore helices, possibly causing the larger degree of tilting that has been observed previously. We should note here that a 2.99 Å crystal structure has been obtained, which shows the anesthetic/analgesic drug ketamine binding to a GLIC structure with binding sites situated between extracellular subunits,⁹ which is consistent with our observations for fentanyl. Fentanyl remains deeper in the intersubunit site compared to ketamine, most likely due to the size of the fentanyl molecule. However, during our simulations, fentanyl was seen to interact with 154-Asp, which is the residue that contributes to the stabilization of ketamine. Ketamine is similar to fentanyl in that it can act as both an analgesic and an anesthetic,^{35,36} which suggests that these molecules may have independent binding sites, compared to those of general anesthetics, in the context of the GLIC structure.

In conclusion, we have presented a novel binding site for the opioid analgesic/anesthetic fentanyl, which, to the best of our knowledge, is the first published evidence of fentanyl

interacting with and modulating the conductance state of GLIC. Our simulations have shown that fentanyl binding induces the closure of the helix pore by causing helix rotation and curvature with minimal tilting, leading to the 233-Ile and 240-Ile residues forming a hydrophobic gate blocking the pore conductance, which is behavior that is similar to that identified for general anesthetics. However, with the use of the TRAJELIX module,²⁵ we were able to identify a rotational motion of the helices that is not observed for anesthetic binding. Discovery of this modulation of GLIC by fentanyl should stimulate further investigations at an atomic level into the role of opioid analgesics in general anesthesia to provide a more complete description of the mechanisms of general anesthesia.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.9b00881.

Methodology, Tables S1–S9, and Figures S1–S10 (PDF)

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C.F., D.F.P., and N.H.d.L. designed the research. C.F. carried out the simulations and analysis. C.F. and N.H.d.L. wrote the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

GLIC, *Gloeobacter violaceus* ion channel; MD, molecular dynamics; TMD, transmembrane domain; MMPSA, molecular mechanics Poisson–Boltzmann surface area; RMSD, root-mean-square deviation; DOPC, dioleoyl-*sn*-glycero-3-phosphocholine.

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